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MOLECULAR DIAGNOSIS OF AETIOLOGICAL AGENTS OF CULTURE-NEGATIVE MENINGITIS

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Rapid and accurate diagnosis is crucial for effective treatment of bacterial meningitis. Bacteriological culture of cerebrospinal fluid (CSF) and blood is still the single most important laboratory investigation which is carried out. However, in some cases, bacteriological culture is unable to detect any causative organisms. This may be due to a number of reasons such as prior antibiotic therapy or, the causative agent may be extremely fastidious.

In this study, we investigated 400 culture-negative CSF samples from patients with suspected acute meningitis by employing 16S rRNA genes, as targets for DNA amplification technologies using the polymerase chain reaction (PCR). Five methods for DNA extraction were compared and the Qiagen Blood Kit proved to be the most reliable and effective. Using a eubacteria PCR method based on the amplification of a 216bp fragment of the 16S rRNA gene, all CSF samples were screened. Contamination was strictly controlled during DNA extraction and PCR amplification in order to avoid false positives. PCR products were sequenced using cycle sequencing on a 373 Applied Biosystems Sequencer.

After PCR amplification, 130/400 (33.5%) were positive. Forty six PCR products subsequently sequenced suggested the presence of DNA from the following: 2/46 *Propionibacterium* spp. (4.34%); 3/46 *Acinetobacter* spp (6.52%); 2/46 *Klebsiella pneumoniae* (4.34%); 2/46 *E. coli* (4.34%); 3/46 *Staphylococcus* spp (6.52%); 3/46 *Haemophilus influenzae* (6.52%); 7/46 *Neisseria meningitidis* (15.22%); 1/46 *Burkholderia* spp (2.17%); 10/46 unidentified bacterium and 14/46 mixed (30.43%).

In conclusion, such molecular based technologies may be used to ascertain the identity of the causal agents of culture-negative meningitis thereby leading to the earlier administration of appropriate chemotherapy to the patient and to ascertain the need for prophylaxis and vaccination of contact cases.

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FATAL PULMONARY EMBOLISM IN A TEENAGE GIRL: A CASE REPORT

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A 19 year old female student was unwell for one week with symptoms of a respiratory tract illness. While being seen by General Practitioner she developed cardiorespiratory arrest. Attempts at resuscitation were unsuccessful. Post-mortem examination revealed bilateral pulmonary thromboemboli and pulmonary infarction with free and organised thrombus of the pelvic veins. Histology showed inflammatory myocardial changes which are discussed. Risk factors for thrombus formation such as smoking or obesity were not present. The only risk factor for thromboembolism identified was oral contraceptive use. Studies have shown that oral contraceptives increase the incidence of venous thrombosis and embolism three to four fold. This is presumed to result from their effects on the haemostatic system. Increased activity of coagulation factors, enhanced platelet activity and a reduction in antithrombin III levels have all

been observed with pill usage. An inherited thrombophilic disorder is not uncommonly found among patients with venous thrombosis, and in oral contraceptive users may increase the risk of thrombosis as much as thirty to fifty fold. A thrombophilia screen of family members undertaken revealed a slightly prolonged activated partial thromboplastin time in female family members with no inhibitor present. Further studies showed a reduction in factor XII levels below the normal range. Otherwise the thrombophilia screen was normal. We conclude that mild reduction of Factor XII levels may constitute a previously unrecognised risk factor for thrombembolic disease in contraceptive pill users.

PCR DETECTION OF FUNGAL AGENTS IN THE IMMUNOCOMPROMISED AND IMMUNOCOMPETENT HOST

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Laboratory culture and serological methods continue to be the diagnostic cornerstone in the detection of medically important fungi in a variety of disease states, including endocarditis, meningitis, febrile neutropenia, diabetes, cystic fibrosis. However fungi are difficult to culture *in vitro* and therefore present diagnostic problems under such circumstances. Employment of the nuclear rDNA genes encoding the 5S, 18S, 5.8S and 28S rRNA offers a molecular basis for both the detection and the identification of fungi.

The aim of this study was (i) to ascertain a suitable method to extract yeast/fungal DNA from various fluid and tissue samples from patients with suspected fungal infections, (ii) to detect and identify these microbiological agents by amplification of various ribosomal target gene loci i.e. small ribosomal subunit (18S rRNA), large ribosomal subunit (28S rRNA), 5.8S rRNA and interspacer regions ITS1 and ITS2, using PCR and direct sequence analysis, (iii) to separate and identify multiple fungal agents in a single clinical specimen.

Twenty five medically important fungi were analysed by PCR amplification and sequence analysis in order to ascertain that the ITS region was the most suitable for detection and accurate identification.

The optimum method demonstrated included an initial DNA extraction method comprising treatment of the specimen with lyticase followed by extraction with proteinase K, guanidine hydrochloride. It was noted that care should be taken in extracting BacTAlert blood culture material, as it was shown that this material was intrinsically contaminated with DNA from both *Lactococcus lactis* and *Saccharomyces cerevisiae*. Primer selection indicated was the ITS1 and ITS2 regions for detection and the 5.8S – ITS2 region for sequence identification. Where sputa are shown to contain several mixed fungal genera and species, it is recommended that each species be separated on a high-resolving acrylamide gel (ExcelGel 48S, Pharmacia), before excision and simple elusion, reamplification and sequence analysis of single clones.

In conclusion, this optimised method may allow for a better understanding of fungi in infection and help in deciding the most appropriate management of the patient.

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ERYTHROPOIETIN RESPONSE TO TRAUMA

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Erythropoietin (EPO) is essential for the production of red blood cells. However in a number of conditions the normal relationship between anaemia, hypoxia and EPO production is not so clearly defined and EPO response may be inhibited. The aim of this study was to investigate the EPO response to trauma, and its variation with time and degree of injury.

Eighteen trauma patients admitted to The Intensive Care Unit of The Royal Victoria Hospital were studied. All had an Injury Severity Score (ISS) ≥16, and were enrolled within 16 hours of injury. Blood samples were taken every

four hours for 24 hours, every eight hours for the next 48 hours and daily thereafter until day 7. The samples were spun, aliquoted and frozen to – 70°C within one hour of sampling for subsequent EPO, Interleukin-6 (Il-6) and soluble tumour necrosis factor receptor – p55-measurements.

Two patient groups were defined with ISS scores 25 and 25 respectively and were otherwise well matched.

Both levels of injury severity resulted in significantly elevated levels of erythropoietin ($p<0.05$). The more severely injured group had an initially higher level of EPO, with the less severely injured reaching this level within 24 hours. The median concentration of EPO in either group did not reach a therapeutic range at any time. Both groups demonstrated significantly elevated levels of both p55 and IL-6, with no significant difference between ISS groups. The three patients who died from multiple organ dysfunction (MODS), revealed initial elevated EPO levels which were significantly higher than the levels seen in those patients who survived. This initially high EPO concentration decreased towards normal within 24 hours. In conclusion trauma appeared to blunt the normal EPO response; however there was no correlation with degree of injury.

ADVERSE HISTOPATHOLOGICAL FEATURES IN CERVICAL LESIONS AS PREDICTORS OF HIGH RISK HUMAN PAPILLOMA VIRUS INFECTION

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Human Papilloma Virus (HPV), which is the main aetiological agent in cervical cancer has been found to induce a number of cytopathic effects in cervical squamous cells. Koilocytic change has long been recognised as an indicator of low risk HPV infection in pre-malignant and malignant lesions. We examined a number of histopathological features and their relationship with high risk HPV infection.

DNA was extracted from a total of 83 microdissected archival cervical lesions using single step proteinase K digestion. The HPV status of the lesions was determined by PCR using generic primers for HPV types 16, 18, 31, 33, 52, 58, 6 and 11 and type specific primers for HPV 16 and 18. HPV PCR products were confirmed by restriction enzyme digestion. Haematoxylin and eosin stained sections were reviewed for the presence of six histopathological features some of which have been previously shown to be associated with microinvasive cancer. Bivariate analysis was used to determine the relationship between the presence of adverse histopathological features and infection with HPV 16 and 31.

All of the histopathological features examined were shown to have a strong statistical relationship with grade of lesion. Absence of koilocytic change was a feature of high grade and invasive lesions. Statistical significance was found between infection with HPV 16 and 31 and all of the histopathological features.

The absence of koilocytic change and the identification of histopathological markers particularly intralesional squamous maturation, comedo necrosis and apoptosis in cervical lesions should be seen as indicators of the presence of high risk HPV types and therefore a potential for progression.

PYREXIA OF UNKNOWN ORIGIN

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Pyrexia of unknown origin remains a common diagnostic challenge for both the clinician and histopathologist. We report the case of an elderly female patient admitted to a district general hospital with rectal bleeding due to over anticoagulation with warfarin. She had many medical problems and described a six month history of nausea, weight-loss, lethargy and sweats. As an-inpatient she had persistent pyrexia despite antibiotic cover. Extensive investigations failed to establish a diagnosis and a bone marrow aspirate and trephine biopsy were performed. A subsequent Ziehl-Neelsen stain on sections of the trephine biopsy revealed the presence of acid-fast bacilli. Anti-tuberculous therapy was instituted and her pyrexia settled over a four week period. Unfortunately she later succumbed to cardiac and renal failure. *Mycobacterium tuberculosis* remains the leading cause of mortality worldwide with three million deaths per year, many cases being diagnosed at autopsy.

Bone marrow examination remains a most useful investigation in patients with pyrexia of unknown origin.

CORRELATION OF DNA PLOIDY, GRADE AND OTHER PROGNOSTIC PARAMETERS IN 156 CASES OF BREAST CARCTNOMA

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We present a retrospective study of 156 patients with breast cancer and correlate the following parameters: ploidy, histological type, histological grade, estrogen receptor, lymph node status, and tumour size.

All the data was collected from the Department of Histopathology at University College Hospital, Galway, between 1996 and early 19999. 83 cases showed a DNA diploid pattern (53.2%) and 73 cases showed a DNA aneuploid pattern (46.7%).

We found a strong correlation between ploidy and the histological grade of the tumour ($p<0.001$). However, no correlation was found between ploidy and the other parameters.

RHABDOMYOSARCOMA MIMICKING AN ACUTE HAEMATOLOGICAL MALIGNANCY

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A previously fit 17-year-old male was admitted feeling generally unwell with a purpuric rash. Examination revealed sternal tenderness, hepatomegaly and a right pleural effusion. Initial investigations confirmed thrombocytopenia (19 x 10^9/l) with an otherwise normal blood count. Hypercalcaemia (2.87 mmol/1), elevated liver function tests and a markedly raised LDH level (5166 u/1) were also found.

Peripheral blood film inspection showed the presence of blasts and nucleated red cells while clumps of small round cells were demonstrated on pleural fluid aspiration. A bone marrow biopsy was carried out to confirm our suspicion of acute leukaemia. Aspirate showed clumps of abnormal cells, and examination revealed a solidly hypercellular core packed with small round cells. LCA, CD3 and CD79a markers were negative disproving our primary diagnosis. CAM 5.2 chromogranin, NSE + MIC2 stains were also negative although the Desmin stain was strongly positive giving a diagnosis of rhabdomyosarcoma.

His condition deteriorated due to disseminated intra vascular coagulation and a right haemothorax, despite treatment with IV ifosphamide, doxorubicin, and hydrocortisone. A thoracotomy was required and multiple pleural and pericardial tumour deposits were noted along with a soft tissue mass arising from the chest wall. He died two weeks from presentation.

Extensive marrow infiltration by rhabdomyosarcoma is a rare phenomenon presenting like an acute leukaemia in a handful of reported cases. The differential diagnosis of small round cell tumours includes leukaemia/ lymphoma, peripheral neuroectodermal tumours (PNETS), Ewing's or other sarcomas and neuroblastomas. This case illustrates the need for marker studies to enable a correct diagnosis of these aggressive tumours to be reached.

GRANULOCYTIC SARCOMA PRECEDING A DIAGNOSIS OF ACUTE MYCLOID LEUKAEMIA (M0)

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In July 1998, a previously fit and well 35-year-old male presented to a local hospital with bilateral inguinal lymphadenopathy. He was otherwise asymptomatic with no sweats or weight loss. Biopsy of an excised node showed complete effacement of the normal lymph node architecture with a diffuse infiltrate of large cells. The appearances were felt to be in keeping with a T-cell Non-Hodgkins Lymphoma. A staging CT scan revealed a small group of nodes in the para-aortic and iliac areas; full blood picture and bone marrow biopsy were normal. The patient was commenced on standard 'CHOP' chemotherapy and CT scanning following the third cycle of CHOP showed shrinkage of all nodes to <1 cm. The patient completed 6 cycles of CHOP and was placed on regular review thereafter.

In April 1999, one month post final chemotherapy cycle, the patient complained of night sweats and again swelling in the inguinal area. Further biopsy of an inguinal node again showed total effacement

of the nodal architecture with lymphoid cells, negative for B-cell markers, but focally positive for CD3. It was felt the features were similar to the previous biopsy and again peripheral T-cell lymphoma was diagnosed. The patient was referred to Belfast City Hospital for salvage therapy.

On admission, a full blood picture revealed the patient to be leucopenic with 13% 'blasts' in the peripheral blood. An immediate bone marrow biopsy was performed and this revealed that 84% of the aspirate consisted of blasts. Immunophenotyping showed the blasts to be Sudan black & ANAE negative, but positive for TdT, LCA, HLA-Dr, CD34 and CD33. It was felt this represented an AML M0, preceded by a granulocytic sarcoma which had been mix-diagnosed as Non-Hodgkins Lymphoma. This was confirmed by further staining of the original lymph nodes – now found to be positive for TdT and CD34.

This case is unusual in that development of AML M0 was preceded by a granulocytic sarcoma 9 months previously. It illustrates the pathological pit-falls in the diagnosis of this difficult condition when it occurs without concomitant blood disease. To our knowledge, this is only the second case of granulocytic sarcoma preceding AML M0 to be reported.

MOLECULAR STUDIES IN LYMPHOID MALIGNANCIES

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Immunophenotyping is essential in the study of chronic lymphoproliferative disorders (CLPD) and has led to improved classification and to more effective treatment. Cytogenetic and molecular studies offer a similar ability to define subcategories but are not widely applied.

PCR may be used to demonstrate immunoglobulin heavy chain (IgH) and T-cell receptor (TCR) gene rearrangements. IgH and TCR are coded by several different gene segments which in the germline are widely separated, but become juxtaposed during rearrangement allowing the amplification of a region by PCR. IgH and TCR rearrangements represent unique clonal markers and their study may therefore be used to assess clonality in lymphoid malignancies. Primers have been designed and methods established for the detection of clonal TCRß (partial and complete), TCRγ and IgH (frameworks 3, 2a and 2b) gene rearrangements.

Similar methods have been established for detection of chromosomal translocations commonly found in subcategories of CLPD including t(14;18), t(8;14), t(11;14) and t(2;5). These molecular methods have the potential to make a major contribution in the diagnosis of follicle centre NHL, Burkitt's NHL, mantle cell NHL and anaplastic large cell lymphomas, respectively.

These methods are equally applicable to the study of DNA extracted from peripheral blood or bone marrow cells, bone marrow trephines or formaldehyde-fixed, paraffin wax embedded tissue biopsies. They can be used to confirm clonality, although unlike immunophenotyping are not lineage specific.

Additionally, with advances in the treatment of patients with chronic lymphoid malignancies, they will have an increasing role in the detection of minimal residual disease post-treatment and in the detection of residual tumour in peripheral blood stem cell harvests. Used in conjunction with conventional techniques such as morphology, cytochemistry and immunophenotyping they can provide clinically relevant diagnostic and prognostic information and could lead to proposals for MIC-M classifications of chronic lymphoid malignancies.